73163/001.625

Docket 14255.01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Name Inventor: Anna SYLVAN		Confirmation No. 2027
Application No.:	10/085,774	Group Art Unit: 1634
Filing Date:	February 27, 2002	Examiner: Goldberg, J.A.

Title: METHOD FOR DETERMINING ALLELE FREQUENCIES

DECLARATION UNDER RULE 132

I, PÅL NYRÉN, a Swedish citizen of Riksradsvägen 67, S-128 39 Skarpnäck, Sweden, hereby declare as follows:

1. I am professor in Biochemistry at Kungl. Tekniska Högskolan (KTH); the Royal Institute of Technology, Stockholm, Sweden. I have worked extensively in the fields of bioenergetics, biomembranes, bioluminescence and molecular and cell biology. A copy of my CV is appended as Annex 1.

2. I was a founder member of the company Pyrosequencing AB of Uppsala, Sweden, now named Biotage AB, and a member of the Board of Directors from 1997 to 1999. I presently hold stock in the company Biotage AB.

3. I developed a method for continuous monitoring of pyrophosphate release based on the enzymes ATP sulphurylase and luciferase termed ELIDA (Enzymatic Luminometric Inorganic Pyrophosphate Detection Assay) (Nyrén and Lundin, Anal. Biochem., 151, 504-509, 1985). I have further contributed to the development of a

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DNA sequencing method based on detecting nucleotide incorporation by detecting pyrophosphate release using the ELIDA method (termed "Pyrosequencing"™). I have worked with Pyrosequencing™ for a number of years and have published widely in this area. As my CV also shows, I have supervised a number of PhD students in this area. I am very familiar with and experienced in Pyrosequencing™ technology. A Pyrosequencing™ procedure for sequencing DNA or detecting single base changes is described in WO 98/28440 (hereinafter "Nyrén-2").

4. I am the sole inventor of the invention described in WO 98/28440 (Nyrén-2). It has been explained to me that Nyrén-2 has been cited against the present application. In particular, I understand the Examiner considers that Nyrén-2 renders the present invention obvious.

5. Nyrén-2 is concerned only with sequencing methods i.e. methods of determining base sequence information, whether about a stretch of nucleotides in a sequence or of a single base. Nowhere does Nyrén-2 in any way suggest that the method disclosed may have a utility beyond sequencing and sequencing-related applications such as detection of single base changes. In such methods of sequencing or base detection a single homogenous sample is used (see Nyrén-2 at page 18, lines 13-26). Nyrén 2 does not suggest pooling of different samples. As discussed further below, the level of accuracy of quantification required to detect the frequency of an allele within a pooled sample is much higher than that required to detect the presence of heterozygous alleles in a single sample. The disclosure of Nyrén-2 at page 18, lines 13-26 concerns detecting whether a sample is heterozygous or homozygous, i.e. whether or not a heterozygote is present. This is not the same as quantification of the precise amount of heterozygous material present; there is no quantification of the alleles present and allele frequencies are not determined. Although I have worked extensively in the field of Pyrosequencing™, it did not occur to me that Pyrosequencing™ could be used on pooled samples, for example to detect or analyse single nucleotide polymorphisms (SNPs) in pooled samples or for any form of allele quantification study. I did not consider using the Pyrosequencing™-based detection/sequencing methods disclosed in Nyrén-2 for

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any other purpose than obtaining sequence information or detecting the presence of a particular base. Further, I do not believe that Nyrén-2 provides a skilled worker in the field with any motivation for using Pyrosequencing^{TM} for any further purpose, particularly for determining allele frequency in pooled samples.

6. Quantification methods on pooled samples such as allele frequency determinations, are very demanding applications which require very high levels of accuracy, much more so than in simple sequencing or base detection. It is of the utmost importance that the method used gives accurate quantitative data on the frequency of each allele. Detection of genetic changes such as SNPs or loss of heterozygosity in pooled samples requires DNA analysis methods capable of highly accurate and reliable quantitative analysis. Thus, allele frequency determination or allele quantification requires a method which has a high detection sensitivity and specificity, for example to enable the performance of single nucleotide polymorphism (SNP) studies in pooled samples.

7. In the case of determining whether a sample is homozygous or heterozygous, the method used to detect the alleles does not have to be quantitative, and if it is a quantitative method, it does not need to be highly accurate or exact. Taking as an example the SNP-analysis of a sample with either base A or G in a specific position, for a single individual there will be three different possibilities: two A alleles, two G alleles, or one A and one G allele. With a highly accurate quantitative method the result from an SNP-analysis would be: 100 % A; 100% G; or 50% A plus 50% G. If 100 individual samples are pooled with 50% having only allele A, and 50% having both alleles, such a quantitative method would indicate 75% allele A and 25% allele G. A non-exact quantitative method is good enough to determine if a single SNP is a homozygote (both alleles have the same base) or heterozygote (one of each allele); the signals obtained differ enough to distinguish between the two situations (a background signal of around 20% can be tolerated). However, for pooled samples the non-exact quantitative method might indicate that 20% have allele G and 80% have allele A even if there is no allele G in the sample (a 20% background signal cannot be tolerated). Thus, as indicated above, a quantitative

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allele analysis (e.g. allele frequency determination) in a pooled sample requires a much more highly accurate and precise method of quantitation than simply detecting whether a sample is heterozygous.

8. Up until the present invention, it was not considered in the art that methods of sequencing or base (e.g. polymorphism) detection would be suitable for performing such a sensitive assay (e.g. in an allele frequency determination context), since the methods are not reliable or consistent enough, or do not give sufficiently quantitative discrimination in a pooled sample. Methods such as traditional Sanger sequencing with dye-labels are not sufficiently accurate and quantitative to perform this method. Whilst the Pyrosequencing™ method of Nyrén-2 has a number of advantages compared to the more commonly used sequencing methods, such as the ability to perform real-time detection, leading to increased speed of sequence analysis, it was not *prima facie* apparent, and is not from the disclosures of Nyrén-2, that the Pyrosequencing™ method would be sufficiently quantitative to enable its use in allele frequency determination or allele quantification studies to accurately detect allele frequencies in pooled samples. Given the inaccuracy involved with, and the unsuitability of, other well known and accurate sequencing methods, it was far from obvious that this approach would be quantitative enough to be useful for such applications. It would not have been obvious to a person skilled in the art that the Pyrosequencing™ method (e.g. as disclosed in Nyrén-2) would have been sufficiently quantitative or accurate enough to be used in allele frequency determinations or in pooled samples.

9. There was no indication, and none is provided by Nyrén-2, that Pyrosequencing™ would be appropriate or especially well-suited for highly quantitative analysis and studies. Accordingly it could not have been predicted that the method would be quantitative enough accurately to detect allele frequencies in pools of samples. It was surprising that the Pyrosequencing method of Nyrén-2 worked as well as it did on a quantitative level. It was unforeseen that the Pyrosequencing™ approach would provide sufficiently quantitative data that would correlate sufficiently well to the allele frequencies to enable allele discrimination

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and quantification. It is therefore my opinion that the present application represents a surprising advance in the art and is not in any way obvious over the disclosures of Nyrén-2.

10. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Codes, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Pål Nyrén

PÅL NYRÉN

13/5 - 2005

Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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ANNEX 1

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Bilaga 2
Pål Nyrén

Curriculum Vitae

Date of Birth: March 28th, 1955
Place of Birth: Stockholm, Sweden
Nationality: Swedish
Residence Address: Riksrådsvägen 67
S-128 39 Skarpnäck, Sweden
Phone: +46 8 6498361
Professional Address: Department of Biotechnology
Albanova University center, KTH, The Royal Institute of Technology
SE-106 91 Stockholm, Sweden
Phone: +46 8 55378392 Fax: +46 8 55378468
Mobile: 070-3971630
Email: nyren@kth.se

Academic merits: M.Sc. (Civ ing), Chemical Engineering 1981
Kungl. Tekniska Högskolan, KTH; The Royal Institute of Technology

Ph.D. (Tekn. doktor) Biochemistry 1985
University of Stockholm

Post-doctoral studies 1985/86
LMB, MRC, Cambridge, G.B. (John Walker)

Associate Prof. (Docent), Biochemistry, 1988
University of Stockholm

Professor in Biochemistry, 1999-12-09
Department of Biotechnology, Kungl. Tekniska Högskolan, KTH; The Royal Institute of Technology

Present position: Professor in Biochemistry
Kungl. Tekniska Högskolan, KTH; The Royal Institute of Technology

Research activities: Bioenergetics, Biomembranes, Bioluminescence, Molecular biology and Cell biology, Environmental toxicology
more than 150 publications:
96 articles and 63 abstracts (see Scientific output below).

Patents: 7 filed patent applications
2 approved patents

Postdoctoral research: 1985/86 postdoctoral fellow, LMB, MRC, Cambridge, G.B. (John Walker).

Teaching experience: Lecturer and tutor, Univ. of Stockholm 1980-90.

Lecturer, The Royal Institute of Technology, Stockholm. 1990-
Responsible/examiner and administrator for several courses.

Teaching experience cont.: I have co-supervised the following Ph.D. students: Åke Strid, Beston Nore, Gaza Salih, Yoko Sakai-Nore
I'm the principle supervisor for the following Ph.D. students:
Mostafa Ronaghi (**defended his thesis October 1998 Titel: Pyrosequencing: a tool for sequence-based DNA analysis**),

Samer Karamohamed (**defended his thesis September 1999 Titel: Pyrosequencing: enzymes and assays**), Tommy Nordström(**defended his thesis June 2003 Titel: Pyrosequencing: automation, optimization and template preparation**), Baback Gharizadeh (**defended his thesis November 2003 Titel: Method development and applications of Pyrosequencing technology**), Jonas Eriksson (**defended his thesis Mars 2004 Titel: Advancements in firefly luciferase-based assays and Pyrosequencing technology**), Nader Nourizad,(**defended his thesis May 2004 Titel: Recombinant enzymes in Pyrosequencing technology**) and Anna Svantesson.

Examiner (opposer) at public defence of Ph.D. thesis	Maritha Mendel-Hartvig, UU, 2002
Member of the examination committe at public defence of Ph.D. thesis	More than tweenty different occasions: Ghasem Nuranis SU, Lena Andersson KTH, Anders Hedrum.KTH, Thomas Leitner KI, Christian Orrenius KTH, Catharina Lavebratt KTH, Mats Holmquist KTH, Staffan Tjus SU, Ivar Virgin SU, Agneta Norén SU, Anders Lindblad SU, Annelie Elvängs, SU, Magnus Larsson, KTH, Cecilia Williams, KTH, Magnus Doverskog, KTH, Lena Gumaelius, KTH, Jenny Ottosson, KTH, Katarina Lindroos, UU, Mario Curcio, KTH, Henrik Wernérus, KTH, Anders Schultz, SU, Anna Blomstergren, KTH, Åke Norberg, KI, Henrik Aspeborg, KTH,Maria Boström, KTH.
Professional service	Member of HUGO (Human Genome Organization), 1996- Member of the International Society for Bioluminescence and Chemiluminescence 1996- Member of the Board of Directors: KTH, Biochemistry and Biotechnology 1997- Pyrosequencing AB 1997-1999 The Swedish Chemical Society, Stiftelsen kemi och Kemiteknik 1995-2001 Local committe for Stockholm Graduate School in Biomedicine 1996-2000
Industrial exeriences	Member of the Board of Directors: Pyrosequencing AB (Biotage AB) (Uppsala), 1997-99. Member of the Board of Directors: Magnetic Biosolutions Sweden AB (2002-).
Grants for Scientific Research from:	NFR (National Science Foundation), TFR (Swedish Council for Engineering Research), PyroSequencing AB, Carl Tryggers Stiftelse för Vetenskaplig Forskning, Magnus Bergvalls Stiftelse, Riksbankens Jubileumsfond, Axel och Margaret Ax:son Johnsons Stiftelse för allmännyttiga ändamål, Åke Wibergs Stiftelse, Procordia/Pharmacia AB forskningsstipendium, KTH Profilpotten, NUTEK (the Swedish National Board for Industrial and Technical Development.

Other merits

Founder of the company Biotage AB (former Pyrosequencing AB) Uppsala Sweden.
Price winner (first price) **Innovation cup 1998**.

Research group

Two Ph.D. students.

Scientific output

1. Yu.A. Shakhov and P. Nyrén (1982) A sensitive and rapid method for determination of pyrophosphatase activity. Acta Chem.Scand. B 36, 689-694.

2. Yu.A. Shakhov, P. Nyrén and M. Baltscheffsky (1982) Reconsti- tution of highly purified proton-translocating pyrophospha- tase from Rhodospirillum rubrum. FEBS Lett.146, 177-180.

3. Yu.A. Shakhov, P. Nyrén and M. Baltscheffsky (1983) Reconsti- tution of highly purified proton-translocating pyrophospha- tase from Rhodospirillum rubrum. Biokhimiya, 48, 1347-1351.

4. P. Nyrén and M. Baltscheffsky (1983) Inorganic pyrophos- phate driven ATP-synthesis in liposomes containing membrane-bound inorganic pyrophosphatase and F_0F_1 complex from Rhodospirillum rubrum. FEBS Lett. 155, 125-130.

5. M. Baltscheffsky and P. Nyrén (1984) The membrane-bound inorganic pyrophosphatase, in "Information and Energy Transduction in Biological Membranes" (ed. E.Helmreich), Liss publ. New York, 199-207.

6. P. Nyrén, K. Hajnal and M. Baltscheffsky (1984) Purification of the proton-translocating membrane-bound inorganic pyro- phosphatase from R.rubrum. Biochem. Biophys. Acta 766, 630- 635.

7. M. Baltscheffsky and P. Nyrén (1984) The synthesis and utili- zation of inorganic pyrophosphate. In Bioenergetics, L. Ern- ster, ed., in New Comprehensive Biochemistry, 9, A. Neuberger and L.L.M. van Deenen, eds., Elsevier, Amsterdam, 187-206.

8. P. Nyrén (1985) The proton pumping inorganic pyrophosphatase from Rhodospirillum rubrum. Dissertation University of Stockholm.

9. P. Nyrén and A. Lundin (1985) Enzymatic method for continuous monitoring of inorganic pyrophosphate synthesis. Anal.Biochem. 151, 504-509.

10. M. Baltscheffsky, J. Boork, P. Nyrén and H. Baltscheffsky (1985) Some basic properties of photosynthetic energy coup- ling, In Physiologie Vegétable 23, (5), 697-704.

11. Å. Strid, P. Nyrén, J. Boork, and M. Baltscheffsky (1985) Kinetics of the membrane-bound inorganic pyro- phosphatase from Rhodospirillum rubrum chromato- phores. FEBS Lett. 196, 337-340.

12. M. Baltscheffsky and P. Nyrén (1986) Preparation and recon- stitution of the proton-pumping inorganic pyrophosphatase from Rhodospirillum rubrum. Meth. Enzymol. 126, 538-545.

13. H. Baltscheffsky, M. Lundin, C. Luxemburg, P. Nyrén and M. Baltscheffsky (1986) Inorganic pyrophosphatase and the molecular evolution of biological energy coupling in "Molecular Evolution of Life"(eds. H. Baltscheffsky, H. Jörnvall and R. Rigler) Chemica Scripta 26B, 259-262.

14. P. Nyrén, B.F. Nore and M. Baltscheffsky (1986) Studies on photosynthetic inorganic pyrophosphate formation in Rhodo spirillum rubrum chromatophores. Biochem. Biophys. Acta 851, 276-282.

15. P. Nyrén, B.F. Nore and M. Baltscheffsky (1986) Inorganic pyrophosphate synthesis after a short light flash in chromatophores from Rhodospirillum rubrum. Photobiochem. Photobiophys. 11, 189-196.

16. B.F. Nore, I. Husain, P. Nyrén and M. Baltscheffsky (1986) Synthesis of pyrophosphate coupled to the energylinked transhydrogenase reaction in Rhodospirillum rubrum chromatophores. FEBS Lett. 200, 133-138.

17. M. Baltscheffsky and P. Nyrén (1986). Purification and recon- stitution of the proton translocating membrane bound inorganic pyrophosphatase from Rhodospirillum rubrum. In Membrane proteins, ed. by Azzi, Springer-Verlag Heidelberg. 42-48.

18. P. Nyrén, B.F. Nore and M. Baltscheffsky (1986) PPi synthesis after a short flash in chromatophores from R.rubrum. Biol. Chem. Hoppe-Seyler 367, 144.

19. Å Strid, B.F. Nore, P. Nyrén and M. Baltscheffsky (1987) Diethylstilbestrol is an inhibitor of the H^+-PPase but not of the H^+-ATPase of Rhodospirillum rubrum chromatophores. Biochem. Biophys. Acta 892, 236-244.

20. M.J. Runswick, S.J. Powell, P. Nyrén and J.E. Walker (1987) Sequence of the bovine mitochondrial phosphate carrier protein: Structual relationship to ADP/ATP translocase and the brown fat mitochondria uncoupling protein. EMBO J. 6, 1367-1373.

21. M. Baltscheffsky and P. Nyrén (1987) PPi in the energy conversion system of Rhodospirillum rubrum. In Phosphate Metabolism and Cellular Regulation in Microorganisms, (eds. A. Torriani-Gorini, F.G. Rothman, S. Silver, A. Wright and E. Yagil) 260-263.

22. P. Nyrén (1987) Enzymatic method for continuous monitoring of DNA-polymerase activity. Anal. Biochem. 167, 235-238.

23. M. Baltscheffsky, P. Nyrén, Å. Strid and A. Pramanik (1988) Some characteristics of cyclic photophosphorylation in maize bundle sheath chloroplasts. Biochim. Biophys. Res. Comm. 151, 878-882.

24. H. Baltscheffsky, M. Baltscheffsky, M. Lundin and P. Nyrén (1988) Inorganic Pyrophosphate in Cellular Energetics and Evolution. In The Roots of Modern Biochemistry, (eds.Klein- kauf, von Döhren, Jaenicks) 917-922.

25. B. Norling, Å. Strid and P. Nyrén (1988) Conversion of coupling factor 1 from Rhodospirillum rubrum from Ca^{2+}- ATPase into a Mg^{2+}-ATPase. Biochem Biophys. Acta 935, 123- 129.

26. Å. Strid, P. Nyrén and M. Baltscheffsky (1988) DIETHYLSTIL- BESTROL Interactions with membranes and proteins and the different effects upon Ca^{2+}- and Mg^{2+}-dependent activities of the F_1-ATPase from Rhodospirillum rubrum. Eur. J. Biochem. 176, 281-285.

27. P. Nyrén and Å. Strid (1989) Effect of equisetin on energy linked reactions in Rhodospirillum rubrum chromatophores. Arch. Biochem. Biophys. 267, 659-666.

28. Å. Strid and P. Nyrén (1989) Division of divalent cations into two groups in relation to their effect on the coupling of the F_0F_1-ATPase ofRhodospirillum rubrum to the protonmotive force. Biochemistry 28, 9718-9724.

29. B. Norling, Å. Strid, C. Tourikas and P. Nyrén (1989) Amount and turnover rate of the FoF_1-ATPase and the stoichiometry of its inhibition by oligomycin in Rhodospirillum rubrum chromatophores. Eur. J. Biochem. 186, 333-337.

30. Å. Strid and P. Nyrén (1989) The F_1-ATPase from Rhodopseudo- monas blastica. Acta Chem. Scand. 43, 1007-1008.

31. G.F. Salih and P. Nyrén (1989) Determination of the intra- cellular concentration of inorganic pyrophosphate in Rhodo- spirillum rubrum. In Current Research in Photosynthesis (Kluwer Academic Publishers) vol. III, 209-212.

32. Å. Strid and P. Nyrén (1989) Intrinsic uncoupling of the F_oF_1- ATPase dependent on what divalent cation used.In Current Research in Photosynthesis (Kluwer Academic Publishers) vol. III, 177-180.

33. B. Norling, Å. Strid, C. Tourikas and P. Nyrén (1989) F_oF_1- ATPase content in chromatophores of Rhodospirillum rubrum and stoichiometry of oligomycin binding.In Current Research in Photosynthesis (Kluwer Academic Publishers) vol. III, 173-180.

34. M. Baltscheffsky, A. Pramanik, M. Lundin, P. Nyrén and H. Baltscheffsky (1989) Some similarities and differences between bacterial chromatophore, spinach chloroplast and yeast mitochondrial inorganic pyrophosphatases.In Current Research in Photosynthesis (Kluwer Academic Publishers) vol. III, 197-200.

35. Å. Strid and P. Nyrén (1989) The purified F_1-ATPase of Rhodo-pseudomonas blastica is a Ca^{2+}-ATPase.In Current Research in Photosynthesis (Kluwer Academic Publishers) vol. III, 181-183.

36. P. Nyrén, B.F. Nore, G.F. Salih and Å.Strid (1989) Light driven inorganic pyrophosphate synthesis in phototrophic bacteria.In Current Research in Photosynthesis (Kluwer Academic Publishers) vol. III, 23-28.

37. B.F. Nore, P. Nyrén, G.F. Salih and Å. Strid (1990) Photo- synthetic formation of inorganic pyrophosphate in phototrophic bacteria. Photosynth. Research. 24, 75-80.

38. P. Nyrén and Åke Strid (1990) The physiological role of the membrane-bound proton-translocating pyrophosphatase in some phototrophic bacteria. FEMS Microbiol. Lett. 77, 265-270.

39. P. Nyrén, B.F. Nore and Å.Strid (1990) Proton pumping DCCD-sensitive inorganic pyrophosphatase from Rhodospirillum rubrum: Purification, characterization and reconstitution. Biochemistry 30, 2883-2887.

40. B.F. Nore, Y.Sakai-Nore, M.Maeshima, M.Baltscheffsky and P.Nyrén (1991) Immunological cross-reactivity between protonpumping inorganic pyrophosphatases of widely phylogenic separated species. Biochim. Biophys. Res. Comm. 181, 962-967.

41. Y. Sakai-Nore, P. Nyrén and Å. Strid (1992) Amino acid sequence similarities between proton pumping proteins.In Research in Photosynthesis (Kluwer Academic Publishers) vol. II, 693-696.

42. P. Nyrén, Y. Sakai-Nore and Å. Strid (1993) Amino acid sequence similarities between the vacuolar proton-pumping inorganic pyrophosphatase and the c-subunit of FoF1-ATPases. Plant Cell Physiol. 34, 375-378.

43. P. Nyrén, B. Pettersson and M. Uhlén (1993) Solid phase DNA mini-sequencing by an enzymatic luminometric inorganic pyrophosphate detection assay. Anal. Biochem. 208, 171-175.

44. P. Nyrén and M. Uhlén (1993) A Method for solid phase DNA sequencing by an enzymatic inorganic pyrophosphatase detection assay. English patent application.

45. P. Nyrén (1994) Apyrase immobilised on paramagnetic beads used to improve detection limits in bioluminometric ATP monitoring. J. Biolumin. Chemilumin. 9, 29-34.

46. P. Nyrén (1994) A method for the detection of cells, cell lysis or cell lysing activity. Swedish patent application.

47. P. Nyrén and Viola Edwin (1994) INORGANIC PYROPHOS-PHATASE-BASED DETECTIONS: Detection and enume-ration of cells. Anal. Biochem. 220, 39-45.

48. P. Nyrén and Viola Edwin (1994) INORGANIC PYRO- PHOSPHATASE-BASED DETECTIONS: Detection and quantification of cell lysis and cell-lysing activity. Anal. Biochem. 220, 46- 52.

49. P. Nyrén and Viola Edwin (1994) A general approach for detection and quantification of cells, cell lysis and cell- lysing activity. In Bioluminescence and

Chemiluminescence Fundamentals and applied aspects, ed. by Campbell et al. (John Wiley and Sons) 195-198.

50. P. Nyrén, M. Uhlén, and M. Ronaghi (1996) Method of Sequencing DNA (PPi sequencing). English Patent application.

51. M. Ronaghi, S. Karamouhamed, B. Pettersson and M. Uhlén, and P. Nyrén (1996) Real-time DNA sequencing using detection of pyrophosphate release. Anal. Biochem. 242, 84-89.

52. S. Karamouhamed, M. Ronaghi, and P. Nyrén (1996) A method for real-time detection of the effect of different inhibitors on reverse transcriptase activity In Bioluminescence and Chemiluminescence Molecular reporting with photons, ed. by Hastings et al. (John Wiley and Sons) 279-282.

53. P. Nyrén, S. Karamouhamed, and M. Ronaghi (1996) Real-time sequence-based DNA analyses using bioluminescence In Bioluminescence and Chemiluminescence Molecular reporting with photons, ed. by Hastings et al. (John Wiley and Sons) 466-469.

54. P. Nyrén (1996) Method of Sequencing DNA (apyrase sequencing). English Patent application.

55. P. Nyrén, S. Karamouhamed, and M. Ronaghi (1997) Detection of single-base changes using a bioluminometric primer extension assay. Anal. Biochem. 244, 367-373.

56. M. Baltscheffsky, M. Brosché, T. Hultman, L. Lundvik, P. Nyrén, Y. Sakai-Nore, A. Severin, and Å. Strid (1997) A 3-hydroxyl-3-methylglutaryl-CoA lyase gene in the photosynthetic bacterium Rhodospirillum rubrum. Biochim. Biophys. Acta 1337,113-122.

57. S. Karamouhamed, M. Ronaghi, and P. Nyrén (1997) A bioluminometric method for real-time detection of reverse transcriptase activity. BioTechniques 24, 302-306.

58. M. Ronaghi, M. Uhlén, and P. Nyrén (1998) A sequencing method based on real-time pyrophosphate. Science 281, 363-365.

59. M. Ronaghi, B. Pettersson, M. Uhlén, and P. Nyrén (1998) PCR-introduced loop structure as primer in DNA sequencing. Biotechniques, 25, 876-878.

60. P. Nyrén, M. Ronaghi, S. Karamohamed, T. Nordström, and K. Nourizad (1999) A novel bioluminometric approach for DNA sequencing. In Bioluminescence and Chemiluminescence: Perspective for the 21st Century, ed. by Roda et al. (John Wiley and Sons) 75-78.

61. T. Nordström, M. Ronaghi, P. Nyrén (1999) Automation of a novel DNA sequencing method. In Bioluminescence and Chemiluminescence: Perspective for the 21st Century, ed. by Roda et al. (John Wiley and Sons) 528-531.

62. S. Karamouhamed, J. Nilsson, K. Nourizad, M. Ronaghi, B. Pettersson and P. Nyrén. (1999) Production, purification, and real-time functional analysis of recombinant *Saccharomyces cervisiae* MET3 adenosine triphosphate sulfurylase expressed in *Escherichia coli*. Protein Expression and Purification 15, 381-388.

63. S. Karamohamed, T. Nordström, and P. Nyrén (1999) A real-time bioluminometric method for detection of nucleoside diphosphate kinase activity. Biotechniques 26, 728-734.

64. M. Ronaghi, M. Nygren, J. Lundeberg, P. Nyrén (1999) Analyses of secondary structures in DNA by Pyrosequencing. Anal. Biochem. 267, 65-71.

65. S. Karamohamed and P. Nyrén (1999) Real-time detection and quantification of adenosine triphosphate sulfurylase activity by a bioluminometric approach. Anal. Biochem. 271, 81-85.

66. A. Ahmadian, J. Lundeberg P. Nyrén, M. Uhlén, and M. Ronaghi (2000) Analysis of the p53 tumor supressor gene by pyrosequencing. Biotechniques 28, 1, 140-144, 146-147.

67. T. Nordström, M. Ronaghi, R. Morgenstern, L. Ekström, U. de Faire, and P. Nyrén (2000) Direct analysis of single nucleotide polymorphism on double-stranded DNA. Biotechnol. Appl. Biochem. 31, 107-112.

68. A. Ahmadian, B. Gharizadeh, A. Gustafsson, F. Sterky, U. Uhlén, P. Nyrén and J. Lundeberg (2000) Single nucleotide polymorphism analysis by pyrosequencing. Anal. Biochem. 280, 103-110.

69. T. Nordström, K. Nourizad, M. Ronaghi, and P. Nyrén (2000) Method enabling pyrosequencing on double-stranded DNA. Anal. Biochem. 282, 186-193.

70. M. Nygren, M. Ronaghi, P. Nyrén, J. Albert, J., and J. Lundeberg (2000) Quantification of HIV-1 using multiple quantitative PCR standards and bioluminometric detection. Anal. Biochem. 288, 28-38.

71. C. A. Garcia, A. Ahmadian, B. Garizadeh, J. Lundeberg, M. Ronaghi, and P. Nyrén (2000) Mutation detection by pyrosequencing: sequencing of exons 5 to 8 of the p53 tumor suppressor gene. Gene 253/2, 249-257.

72. P. Nyrén, M. Ronaghi, and A. Tallsjö (2000) Method of Sequencing DNA (inactive isomer). English Patent application.

73. T. Nordström, B. Gharizadeh, N. Pourmand, P. Nyrén, and M. Ronaghi (2001) Method enabling fast partial sequencing of cDNA clones. Anal. Biochem. 292, 266-271.

74. B. Gharizadeh, M. Kalantari, C. Garcia, B. Johansson, and P. Nyrén (2001) Typing of human papillomavirus (HPV) by pyrosequencing. Laboratory Investigation 81, 673-679.

75. J. Eriksson, S. Karamohamed, and P. Nyrén (2001) Method for real-time detection of inorganic pyrophosphatase activity. Anal. Biochem. 293, 67-70.

76. B. Gharizadeh, T. Nordström, A. Ahmadian, M. Ronaghi and P. Nyrén (2001) Long read pyrosequencing using pure 2'-deoxyadenosine-5'-O'-(1-thiotriphosphate) Sp-isomer. Anal. Biochem. 301, 82-90.

77. A. Ahmadian, P. Nyrén, and J. Lundeberg (2001) Allele specific primer extension assay. English Patent application.

78. P. Nyrén, A. Ahmadian, and J. Lundeberg (2001) Allele specific primer extension. English Patent application.

79. T.Nordström, A. Alderborn, and P. Nyrén (2002) Method for one-step preparation of double-stranded DNA template applicable for use with Pyrosequencing technology. J. Biochem. Biophys. Methods 52, 71-82.

80. B. Gharizadeh, Elin Norberg, Jürgen Löffler, Shah Jalal, Jan Tollemar, Hermann Einsele, Lena Klingspor, and P. Nyrén (2004) Identification of medically important fungi by pyrosequencing technology. Mycoses, 47, 29-33.

81. P. Nyrén (2003) Method for bioluminometric assays at elevated temperature. Swedish patent application.

82. J. Eriksson, T. Nordström, and P. Nyrén (2003) Method enabling firefly luciferase based bioluminometric assays at elevated temperature. Anal. Biochem. 314, 158-161.

83. N. Nourizad, M. Ehn, B. Gharizadeh, S. Hober, and P. Nyrén (2003) Methylotrophic yeast Pichia pastoris as a host for production of ATP-diphosphohydrolase (apyrase) from potato tubers (Solanum tubersum). Prot. Express. Purif. 27, 229-237.

84. P. Nyrén, T. Nordström, and B. Gharizadeh (2003) Pyrosequencing technology. In: PCR Technology: Current Innovations (second edition), CRC Press Edited by T. Weissensteiner, H.G. Griffin, A. Griffin, 187-195.

85. B. Gharizadeh, M. Ghaderi, D. Donnelly, K-L. Wallin, and P. Nyrén (2003) Multiple-primer DNA sequencing method. Electrophoresis, 24, 1145-1151.

86. N. Nourizad, B. Gharizadeh, and P. Nyrén (2003) Method for clone checking. Electrophoresis, 24, 1712-1715.

87. X.Y. Zhao, B. Gharizadeh, X.B. Wang, M. Ghaderi, R. Pirskanen, P. Nyrén, and A.K. Lefvert (2004) β2-adrenergic receptor gene promoter SNPs : associations with human myasthenia gravis with thymoma. Submitted.

88. B. Gharizadeh, A. Ohlin, P. Mölling, A. Bäckman, B. Amini, P. Olcén, and P. Nyrén (2003) Multiple group-specific sequencing primers for reliable and rapid DNA sequencing. Mol. Cell. Probe 17, 203-210.

89. B. Gharizadeh, J. Eriksson, N. Nourizad, T. Nordström, and P. Nyrén (2004) Improvements in Pyrosequencing technology by employing Sequenase polymerase Analytical Biochemistry 330/2, 272-280.

90. J. Eriksson, B. Gharizadeh, T. Nordström and P. Nyrén (2004). Pyrosequencing technology at elevated temperature. Electrophoresis 25, 20-27.

91. X.Y. Zhao, B. Gharizadeh, P. Hjelmström, R. Pirskanen, P. Nyrén, A-K. Lefvert, and M. Ghaderi, (2003) Genotypes of CCR2 and CCR5 chemokine receptors in human myasthenia gravis. International Journal of Molecular Medicine 12, 749-753.

92. B. Gharizadeh, M. Käller, P. Nyrén, A. Andersson, M. Uhlén, J. Lundeberg and A. Ahmadian, (2003) Viral and microbial genotyping by a combination of multiplex competitive hybridization and specifik extension followed by hybridization to generic tag arrays. Nucl. Acids Research 31, 22, e146, 1-12.

93. M. Ehn, N. Nourizad, K. Bergström, A. Ahmadian, P. Nyrén, J. Lundeberg and S. Hober (2004) Towards Pyrosequencing on surface-attached genetic material by use of DNA binding luciferase fusion proteins. Analytical Biochemistry 329, 11-20.

94. G. Gambelunghe, M. Ghaderi, A. Brozzetti, P. Del Sindaco, B. Gharizadeh, P. Nyrén, P. Hjelmström, L. Nikina-Zake, C. B. Sanjeevi and A. Falorni (2003) Lack of association of CCR2-64I and CCR5-Δ32 with type 1 diabetes and latent autoimmune diabetes in adults. Hum. Immunol. 64, 629-632.

95. B. Gharizadeh, M. Oggionni, B. Zheng, E. Akom, N. Pourmand, A. Ahmadian, K-L. Wallin and P. Nyrén (2004) Type-specific multiple sequencing primers: a novel strategy for reliable and rapid genotyping of human papillomaviruses by Pyrosequencing technology. Submitted.

96. J. Eriksson, B. Gharizadeh, N. Nourizad and P. Nyrén (2004) 7-deaza-2'-deoxyadenosine-5'-triphosphate as an alternative nucleotide for the Pyrosequencing technology. Nucleosides, Nucleotides & Nucleic Acids, Volume 23, Issue 10, 19-30.

97. S. Svantesson, P.O. Westermark, J. Hellgren Kotaleski, B. Gharizadeh, A. Lansner and P. Nyrén (2004) A mathematical model of the Pyrosequencing reaction system. Biophysical Chemistry 110, 129-145.

98. Gambelunghe G, Ghaderi M, Gharizadeh B, Brozzetti A, Tortoioli C, Del Sindaco P, Sanjeevi CB, Hjelmstrom P, Sirsjo A, Nyren P, Santeusanio F, Falorni A. Lack of association of human chemokine receptor gene polymorphisms CCR2-64I and CCR5-Delta32 with autoimmune Addison's disease. Eur J Immunogenet. 2004 Apr;31(2):73-6.

99. Lindbäck, E., Gharizadeh, B., Ataker, F., Airell, Å., Jalal, S., Nyrén, P. and Wretlind, B. (2004) DNA gyrase gene in *Neisseria gonorrhoeae* as indicator for resistance to ciprofloxacin and species verification. International Journal of STD and AIDS in press.

Abstracts

1. P. Nyrén, Yu.A. Shakhov and M. Baltscheffsky (1982) 2nd Bio-energetic Conference, Lyon. SHORT REPORTS 325.
2. M. Baltscheffsky, P. Nyrén and Yu.A. Shakhov (1982) IV Int. Symp. on Photosynthetic Procaryotes, Bordeaux.
3. P. Nyrén and M. Baltscheffsky (1983) 6th Int. Congr.on Photo- synthesis, Bryssel. Abstracts 1, 271.
4. P. Nyrén, B.F. Nore and M. Baltscheffsky (1986) 4th Bio- energetic Conference, Prague. EBEC reports 4, 129.
5. B.F. Nore, I. Husain, P. Nyrén and M. Baltscheffsky (1986) 4th Bioenergetic Conference, Prague. EBEC reports 4, 327.